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14. ABSTRACT

This research project is a result of a collaboration between three research groups aimed at elucidating basic replication processes of CCHFV with the expected outcome of providing basic research reagents and establishing the foundation of knowledge necessary for discovery of vaccines and antiviral therapeutics for Crimean Congo hemorrhagic fever. Our major findings during the third year of support are the following: We have demonstrated that the isopeptidase activity associated with the N-terminal of the L protein is responsible for overcoming innate immune responses mediated by ubiquitin and by the ubiquitin-like molecule ISG15, and that this activity is shared with the nsp2 of an unrelated virus family: the arteriviruses. This activity could be a target for antiviral development. We have characterized in more detail the Nsm protein of CCHFV and developed constructs with fusogenic activity based on expression of the G ORF. We have successfully passaged CCHFV 18 times in SCID mice and conducted preliminary studies in macaques. Our results provide novel insights on the molecular biology of this understudied highly pathogenic human virus.

15. SUBJECT TERMS
CCHFV, bunyavirus, reverse genetics, virus receptor, animal model, arbovirus

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1. Introduction.

This investigator-initiated project represents a collaborative team approach to the study of a highly pathogenic emerging virus of military importance, Crimean Congo hemorrhagic fever virus (CCHFV). CCHFV causes disease characterized by abrupt onset fever and can progress to hemorrhage, renal failure and shock. Mortality 13-50% is common. This severe disease is endemic in sub-Saharan Africa, the Middle East, and central Asia, all areas of current significant military operations. The extremely pathogenic nature of CCHFV has led to the fear that it might be used as an agent of bioterrorism or biowarfare. This proposal is comprised of three interrelated subprojects aimed at elucidating basic replication processes of CCHFV with the expected outcome of providing basic research reagents and establishing the foundation of knowledge necessary for discovery of vaccines and antiviral therapeutics for CCHF. The body of the progress report for each sub-project is outlined in the following pages.

2. Body.

Subproject #1: Reverse genetics of CCHFV

Subproject #1 focuses on understanding the molecular interactions between the components of the RNA replication machinery of CCHFV: the N and L proteins and the viral RNAs, with the final goal of establishing reverse genetics techniques for the rescue of CCHFV from plasmid DNA. These techniques will be used to generate attenuated strains of CCHFV. There are three tasks:

Task 1. Characterization of cis- and trans-acting signals involved in RNA replication and transcription of CCHFV.

This task included the development of expression plasmids encoding the N and L proteins of CCHFV and model vRNAs encoding reporter genes supporting the replication and transcription of this viral RNA when transfected into cells. These plasmids will be used also under this task to perform mutational analysis to identify sequence/structure regulatory motifs involved in viral RNA replication and transcription, as well as to identify domains involved in protein-protein interactions and protein-RNA interactions.

During the first year, we sequenced and cloned the L (large polymerase) and N (nucleoprotein) open reading frames of CCHFV, strain 10200 into expression plasmids, as well as generated different reporter genes (GFP and luciferase) flanked by the 3' and 5' noncoding regions of the S segment of CCHFV. During the second year, we have mapped domains in N involved in the interaction of this protein with RNA, with itself and with the L protein. In this third year of this project, we have concentrated in studying the unusual protease activity that we identified associated with the N-terminal domain of L. In addition, we have generated new reagents for the more detail mapping of protein-protein and protein-RNA interactions

Co-immunoprecipitation analysis showed that CCHFV-N is able to interact with itself in transfected cells. In order to further explore the number of N molecules involved in these oligomers, we are planning to perform Gel filtration analysis of bacteria-purified CCHFV-N. Since GST-N protein cannot be used for these analyses because of the GST ability to interact

with itself, we sub-cloned CCHFV-N the pET-15b expression vector (Novagen). This recombinant protein expresses a 6xHis tag in its N-terminus.

To map in more detail the domains involved in N-N interaction, we moved to the mammalian two hybrid system (Promega). N(1-482) along with N(1-240), N(1-160), N(240-482), N(160-482) and N(160-240) truncation mutants have been described in our previous progress report. All these constructs, and a N(delta c-c) construct were subcloned into the pACT and pBIND vectors of the mammalian two hybrid system. The N(delta c-c) expresses the N protein in where the coiled-coiled domain was replaced by a 8xGly linker.

We have confirmed the N-L interaction several times (using double- and single-tagged L protein). The next step is to map the domains in N involved in these interaction. Because of the difficulties in subcloning CCHFV-L in the vectors of the mammalian two hybrid system, we will perform the mapping by co-immunoprecipitation assays.

Other functions of the CCHFV replication components. We reported in our previous report the presence of an OTU-like domain at the N-terminus of the L protein of CCHFV, and preliminary evidence for deubiquitinating activity associated with this domain. Ubiquitin and the ubiquitin-like protein ISG15 are critically important in innate immune responses mediated by tumor necrosis factor-alpha (TNF-alpha) and type I interferon (IFN alpha/beta). Ubiquitination regulates NF-kB activation and ISG15 is a potent IFN-induced antiviral molecule. As viruses have evolved immune evasion mechanisms to counteract anti-viral molecules, it is likely that ubiquitin and ISG15 will be targets of immune evasion strategies. The ovarian tumor (OTU) domain is a cysteine protease domain found in eukaryotic, prokaryotic and viral proteins. Although the majority of the OTU-domain containing proteins remain uncharacterized, several OTU-domain mammalian proteins have deubiquitinating (DUB) activity. Viral OTU domains were cloned from the L protein of nairoviruses CCHFV and DUGV, and from the nsp2 protein of arteriviruses EAV and PPRSV. Expression of viral OTU domains resulted in an overall decrease of cellular ubiquitin and ISG15 conjugates. Using deletion mutants from CCHFV-L, the deconjugating activity was mapped to the OTU domain and confirmed by mutating the predicted protease active site residues which restored ubiquitin and ISG15 conjugate levels. In vitro deconjugation assays demonstrated that the CCHFV-L OTU domain could cleave K48- or K63-linked ubiquitin chains and ISG15 conjugates. SUMO-2 or SUMO-3 chains were not deconjugated, suggesting that the CCHFV-L OTU domain may have specificity for certain UB/UBL family members. The physiologic importance of viral OTU domains was assessed in reporter assays, an OTU domain-expressing transgenic mouse and by generating chimeric Sindbis viruses. Transfection of CCHFV-L or EAV-nsp2 OTU domains decreased TNF-alpha induced NF-kB activation. A transgenic mouse expressing the CCHFV-L OTU domain displayed increased susceptibility to Sindbis virus infection. Since expression of ISG15 from a recombinant Sindbis virus increases survival of infected Ifnar-/- mice, we hypothesized that OTU-mediated deISGylation should abolish ISG15's anti-viral function. Co-expression of the OTU domain antagonized ISG15's anti-viral function, while expression of a catalytic mutant OTU domain had no effect on ISG15-mediated protection from lethality. The ability of viral proteins to deconjugate both ubiquitin and ISG15 modified proteins could potentially represent a powerful immunomodulatory mechanism which may allow viruses to affect the regulation of numerous cellular pathways. These results are in press in the new journal Cell, Host and Microbe.

Our characterization of the CCHFV-L OTU domain and the development of in vitro assays for its enzymatic activities will make it feasible to screen for potential inhibitors specific for CCHFV-L and other OTU domain-containing viral proteins. High-throughput screening of chemical compound libraries has proven to be a valuable tool in the identification of small molecule inhibitors of other viral proteases such as HCV-NS3-4A and SARS-3CLpro. Difficulties may arise in trying to develop OTU inhibitors that are specific for the viral proteases and do not affect cellular DUBs. However, since the viral enzymes have a unique capacity to target both Ub and ISG15 conjugates, the design of specific inhibitors might be possible. For this reason, structural studies will be of great value to understand both the molecular basis of the unique biochemical activities of these viral proteins and the potential for development of antiviral compounds.

Task 2. Generation of recombinant CCHFV from cDNA.

In order to improve the efficiency of the minigenome of CCHFV, we have constructed other reporter plasmids besides the previously described pPOLI-S-Luc and pPOLI-S-GFP. The newly made pPOLI-S-Luc #7 and pPOLI-S-CAT encode the reporter genes firefly luciferase or chloramphenicol acetyl transferase enzymes, respectively. These plasmids differ from the pPOLI-S-Luc and pPOLI-S-GFP plasmids in the fact that the reporter genes are directly fused to the S segment UTR (there is no fusion with the S ORF).

We also made reporter genes containing the UTRs of the L segment. We observed that there is a difference at the position 10 in the 3'UTR between our sequence and the sequences deposited by other laboratories (accession numbers NC_005301 and AY389508). These groups reported an A nucleotide in that position (however, this position was not sequenced and assumed to be conserved between the S, M and L segments) and we sequenced a G (Figure 1). We constructed reporter plasmids encoding the luciferase or CAT genes under the control of the 3' UTR sequence as reported by us (pPOLI-L#1-Luc and pPOLI-L#1-CAT) and as reported by the other groups (pPOLI-L#2-Luc and pPOLI-L#2-CAT). All these plasmids have the reporter genes fused to the UTRs.

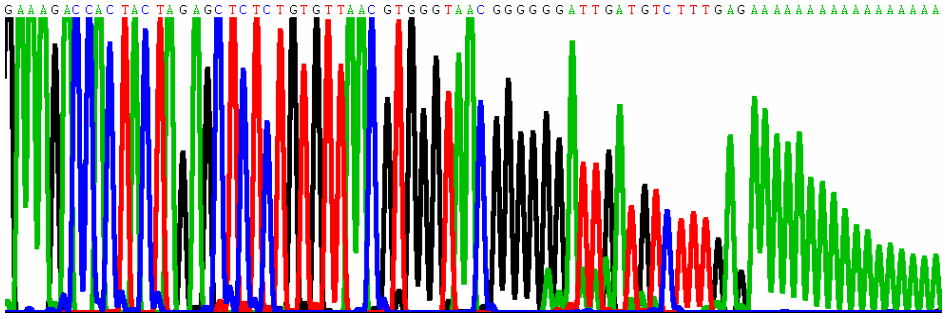


Figure 1. 3'UTR sequence obtained by us (3' end is on the right). Note the presence of a G at position 10.

Task 3. Generation of attenuated CCHFV by reverse genetics. We are currently subcloning the cDNAs of the S, M and L segments into the pT7riboSM2 vector, previously described by Blakqori, G and Weber F (J Virol. 2005 Aug;79(16)). This vector was used by these authors to rescue the bunyavirus Rift valley fever virus.

Subproject #2: Cell Biology of CCHFV Glycoproteins

Work carried out under subproject #2 is being performed in the laboratory of Dr. Robert W. Doms at the University of Pennsylvania, and is directed towards characterizing the envelope glycoproteins of CCHFV. There are two tasks:

Task 1. Analyze the assembly and processing of CCHFV G1 and G2 glycoproteins and characterize the mucin-like domain present at the amino terminus of the polyprotein. (Years 1-3)

Task 2. Identify cell receptors and attachment factors for CCHFV.

Over the three years of this DOD-sponsored project, three papers have been published, two in the Journal of Virology and a second in Journal of General Virology. Two additional papers are being written, as discussed in Section 2. All three published studies were done in collaboration with our colleagues at USAMRIID and Mt. Sinai. Mr. Lou Altamura, the graduate student who leads this project, visits USAMRIID approximately 4 times a year, and spends 1-2 weeks each time to work with live CCHFV in the biocontainment suites. He will graduate in early 2008, and has accepted a postdoctoral position with Dr. Schmaljohn where he will continue to work on CCHFV.

Task 1. Analyze the assembly and processing of CCHFV G1 and G2 glycoproteins and characterize the mucin-like domain present at the amino terminus of the polyprotein. (Years 1-3)

As described in our previous reports, we have developed antibodies and protein expression systems for the CCHFV glycoproteins, and have made considerable progress in studying their synthesis, assembly and transport. Much of our work has been focused on a new protein that we have identified, termed NSm. The CCHFV glycoproteins are derived from the polyprotein precursor encoded by its medium (M) RNA segment through a series of endoproteolytic cleavage events. The M polyprotein is first divided into two intermediate precursors, PreG_N and PreG_C. Stuart Nichol and his colleagues at the CDC have done much to characterize PreG_N precursor processing, showing that the N-terminal mucin-like and GP38 domains being removed by furin and SKI-1 mediated proteolysis, respectively. SKI-1 cleavage generates the N-terminus of mature G_N at amino acid 520 (in strain IbAr10200), and a presumably related protease cleaves the N-terminus of PreG_C to yield a mature G_C beginning at amino acid 1041. However, between Gn and the N-terminus of Gc there is a relatively large stretch of amino acids that contain four predicted transmembrane helices between the G_N and G_C ectodomains. In a paper published this year we showed that the C-terminal region of Gn is cleaved by an as yet unidentified protease, resulting in an NSm protein with a molecular weight of approximately 20 kDa. We found that NSm is produced following M segment expression in a variety of cell types, and importantly found that it is also produced in cells following infection with live CCHFV. We also found that that this protein is produced efficiently and shortly after synthesis of PreGn, that it is stable, and that at least some of it is transported to the Golgi.

We have continued our studies on NSm during the course of this year. If NSm is deleted from the M segment, the Gn and Gc proteins are processed very inefficiently. We do not yet know if this is due to gross structural perturbations resulting from this genetic modification, or if NSm plays a specific role in the folding and assembly process. We have constructed a panel of mutants to explore these possibilities. Efforts have also been made to identify the protease responsible for NSm generation. We screened a very large panel of protease inhibitors, and found that none could inhibit the N terminal cleavage event. This could be because cleavage results from a protease that is not sensitive to the inhibitors we have tested, or because the

inhibitors do not have access to the endoplasmic reticulum, which is where cleavage occurs. To address this, we set up an in vitro translation assay, and found that in the presence of rough microsomes, NSm is produced. Thus, the protease responsible for NSm generation must reside within the ER. However, our panel of inhibitors did not prevent cleavage in this in vitro assay, where presumably accessibility issues should not be significant.

In light of our failure to inhibit proteolysis, we speculate that NSm cleavage may occur within the membrane. Over the past several years, intramembranous proteolysis has been well described. There are several proteases that can cleave peptide bonds within the hydrophobic interior of the membrane, including signal peptide peptidase and presenilin 1 and 2. The presenilins are particularly interesting because they cleave the amyloid precursor protein within the membrane, resulting in the generation of the amyloid peptide that is deposited in plaques that are found within the brains of patients with Alzheimer's diseases. Intramembranous proteolysis is difficult to inhibit with small molecules, so we are taking several approaches. First, we find that the second TM domain of the CCHFV M segment is by far the most conserved of the four TM segments, particularly the C-terminal portion of the domain which is where we predict that cleavage occurs (based on the size of NSm). Second, this domain has several helix-breaking residues that are commonly found in TM domains that are subject to intramembranous proteolysis. Third, a series of point mutants we have made in this domain inhibit NSm cleavage, whereas mutations elsewhere do not. More recently, we have been trying to purify enough NSm to obtain N terminal sequence. This will unambiguously identify the cleavage site, and will likely suggest the protease involved. To do this, we have constructed a series of 'mini-proteins' that contain NSm plus N-terminal flanking regions. Several of these miniconstructs appear to be processed correctly and are secreted from cells, which will make purification easier. For the coming year, we hope to identify the cleavage site, and then introduce specific mutations into intact M segment to confirm that cleavage in the intact protein occurs at the same location. Next, this information will be used to identify the protease responsible for cleavage, and if possible, we will determine if specific protease inhibitors prevent CCHFV replication.

Task 2. Identify cell receptors and attachment factors for CCHFV.

We are finally making real progress in this area. Our fundamental problem has been the lack of a robust cell-fusion assay or virus pseudotype system. The primary difficulty we have faced is that the CCHFV glycoproteins are targeted to the Golgi, and are not delivered to the cell surface. However, Golgi retention mechanisms are generally thought to be saturable, so we reasoned that if we could over-express the Gn and Gc proteins, then some fraction might be delivered to the plasma membrane. In last year's report, we noted that we had gotten a cell fusion assay to work, but at the time results were variable, and signal to noise ratios were relatively low. Fortunately, a new student joined the lab (Lauren Davis) to work on this project, and she has greatly improved the assay to the point that it is working extremely well, and a paper describing this assay is being written.

To assess the ability of the CCHFV glycoproteins to elicit membrane fusion, human 293T cells are transfected with an expression plasmid containing a codon-optimized version of the CCHFV M segment, as well as a plasmid expressing the omega subunit of b-galactosidase. The next day, these cells are mixed with target cells that express the alpha subunit of b-galactosidase. The pH of the media is then adjusted to the desired value for two minutes, after which the cells are returned to normal media for 4 hours. At this point the cells are lysed with a nonionic detergent, and the amount of b-galactosidase activity present is measured with a luminometer.

Fusion between the effector (CCHFV expressing) and target cells results in cytoplasmic mixing and b-galactosidase complementation – neither the alpha nor omega subunits are active on their own. We carry this assay out in deep 94 well plates, which makes this assay reasonably high throughput. We have found that CCHFV fusion is pH dependent, and can occur with a variety of cell types. The importance of this result is that we now have an assay that measures CCHFV glycoprotein function that is not only high throughput, but that can be performed under BSL2 conditions. As a result, we can use this assay to screen for small molecule inhibitors of fusion as well as for neutralizing antibodies. In addition, we have begun a series of structure-function studies in which we have introduced specific mutations into the Gn and Gc proteins in an attempt to identify the roles that each play in the fusion process.

Our goal for year 4 will be to complete our structure function work and submit a paper on this for publication. We will also attempt to develop CCHFV pseudotypes, as these would be a far better tool to use in a search for virus attachment factors and receptors. Now that we have found conditions under which CCHFV Gn and Gc are delivered to the cell surface, we have some hope that this will work. We will attempt to produce pseudotypes with both VSV and retroviral cores, and if this does not work, we will replace the Gc cytoplasmic domain with the corresponding region from the VSV G protein. Such a protein chimera may increase pseudotyping efficiency on VSV cores. If virus pseudotypes can be produced, we will be in a position where a high throughput screen can be performed in an attempt to identify entry inhibitors.

Subproject #3: Characterization of CCHFV strains and small animal model development

Work carried out under subproject # 3 is being performed in the laboratory of Dr. Connie Schmaljohn at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), and is directed at studies involving live virus in high biocontainment and in supporting subprojects 1 and 2 with reagents and experimental verification of in vitro results.

Work during this reporting period has centered on developing animal models for CCHFV. In addition, preliminary work has been performed to optimize gene expression profiling microarrays and to generate a live CCHFV tagged with GFP for verification studies.

Task 1. Determination of genetic factors of virulence by comparison of tick, livestock and human isolates of CCHFV.

Methods:
Profiler PCR Arrays were purchased from SuperArray Biosciences Corporation for use in analyzing the expression of a focused panel of genes for various cytokines and signaling pathways by real time PCR. Each 96-well plate includes SYBR Green-optimized primer assays for interferon or inflammatory pathway-focused genes. These arrays will allow us to monitor the expression profiles of 84 genes per pathway that encode many important immunological molecules.

Results:
Experiments were performed to optimize assay conditions prior to start of real time PCR analysis with the CCHFV infected cells. Studies on the virus infected cells are planned to begin within the next few weeks. These analyses are expected to help define virus-host interactions that

contribute to CCHFV pathogenesis.

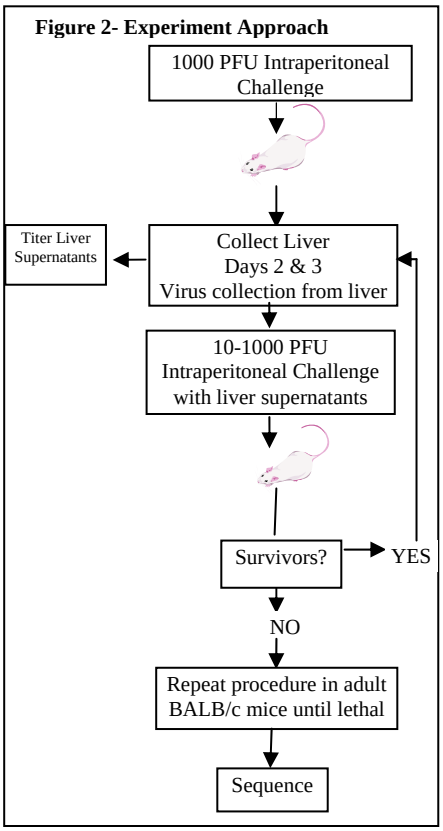
Task 2: Animal model development:

Methods:
1. Development of a mouse model for CCHFV

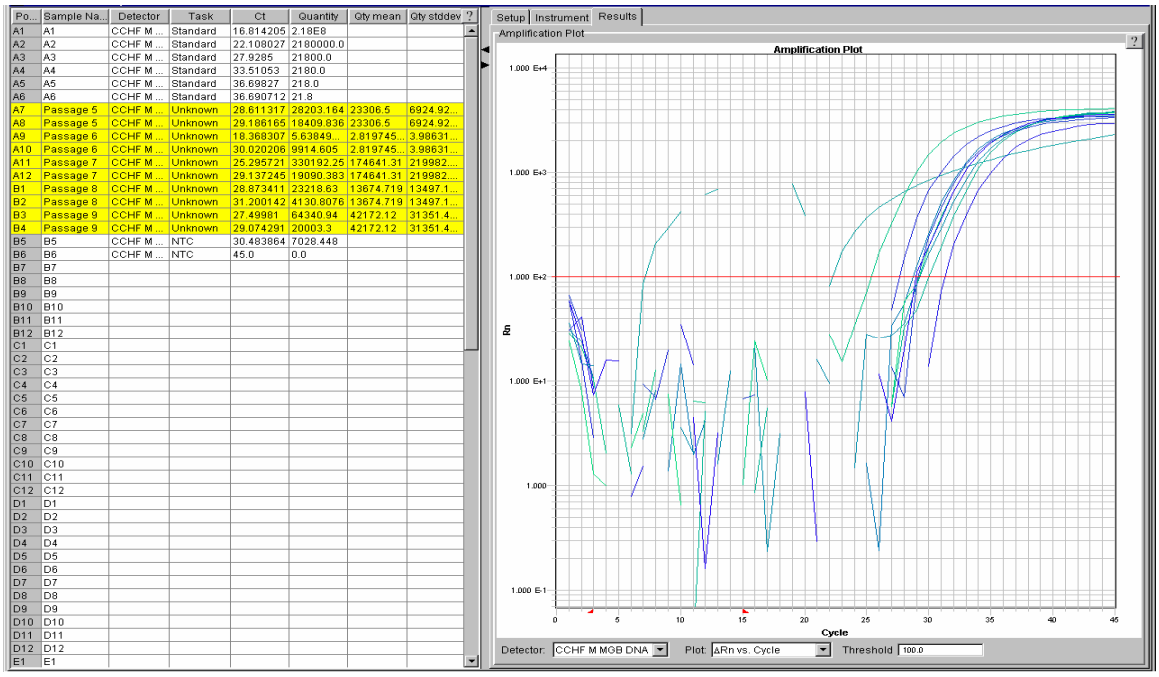
The strategy involves selecting a CCHFV able to kill SCID mice, then further adapting that virus to immunocompetent mice. This method was successfully used in recent studies to adapt the filovirus, Marburg virus, for moue lethality (Warfield, et al., submitted).

Methods:
To date, CCHFV strain 3010, which was isolated from a lethal human case of CCHF in Uganda, has been serially passed 18 times in SCID mice (by intraperitoneal injection of liver homogenates from the previous mouse-passage (Figure 2).

Figure 2- Experiment Approach



Results:
The mice have maintained the virus throughout the passages without symptoms of illness. Virus has been detected by real time PCR in the livers of mice from each passage level to date. An example of the PCR results from passages 5-9 are shown in Fig. 3.



Po...	Sample Na...	Detector	Task	Ct	Quantity	Qty mean	Qty stddev
A1	A1	CCHF M ...	Standard	16.814205	2.18E8		
A2	A2	CCHF M ...	Standard	22.108027	2180000.0		
A3	A3	CCHF M ...	Standard	27.9285	21800.0		
A4	A4	CCHF M ...	Standard	33.51053	2180.0		
A5	A5	CCHF M ...	Standard	36.69827	218.0		
A6	A6	CCHF M ...	Standard	36.690712	21.8		
A7	Passage 5	CCHF M ...	Unknown	28.611317	28203.164	23306.5	6924.92...
A8	Passage 5	CCHF M ...	Unknown	29.186165	18409.836	23306.5	6924.92...
A9	Passage 6	CCHF M ...	Unknown	18.368307	5.63849...	2.819745...	3.98631...
A10	Passage 6	CCHF M ...	Unknown	30.020206	9914.605	2.819745...	3.98631...
A11	Passage 7	CCHF M ...	Unknown	25.295721	330192.25	174641.31	219982....
A12	Passage 7	CCHF M ...	Unknown	29.137245	19090.383	174641.31	219982....
B1	Passage 8	CCHF M ...	Unknown	28.873411	23218.63	13674.719	13497.1...
B2	Passage 8	CCHF M ...	Unknown	31.200142	4130.8076	13674.719	13497.1...
B3	Passage 9	CCHF M ...	Unknown	27.49981	64340.94	42172.12	31351.4...
B4	Passage 9	CCHF M ...	Unknown	29.074291	20003.3	42172.12	31351.4...
B5	B5	CCHF M ...	NTC	30.483864	7028.448		
B6	B6	CCHF M ...	NTC	45.0	0.0		
B7	B7						
B8	B8						
B9	B9						
B10	B10						
B11	B11						
B12	B12						
C1	C1						
C2	C2						
C3	C3						
C4	C4						
C5	C5						
C6	C6						
C7	C7						
C8	C8						
C9	C9						
C10	C10						
C11	C11						
C12	C12						
D1	D1						
D2	D2						
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D4	D4						
D5	D5						
D6	D6						
D7	D7						
D8	D8						
D9	D9						
D10	D10						
D11	D11						
D12	D12						
E1	E1						

Fig. 3

2. Nonhuman primate model development

Methods:

Three cynomolgus macaques were injected intravenously with ~1x10^4 pfu of CCHFV strain 3010 and three others were injected with ~1x10^4 pfu CCHFV strain I248 (isolated from a 1998 lethal human case of CCHFV in Uzbekistan). An additional group of two macaques serve as no virus controls. To date, two weeks post-infection, the animals have not displayed symptoms of illness. Several of the primates have maintained the virus through six days post infection as measured by qPCR. Additional viral load and hematological analyses are ongoing.

Results:
Viral loads through day 12 are currently being assessed.

Task 3: Development of GFP-tagged CCHFV

Methods:

We are attempting to use a Mu A transposon-based mutagenesis system to generate a fusion library in which a GFP encoding cassette is inserted randomly throughout the S RNA segment of the CCHFV genome. Each cDNA clone in the library has a single GFP fusion insert, but taken as a whole, the library has clones containing GFP fused at nearly every position in the S segment. RNA was generated from the fusion library by in vitro transcription, and transfected into 293T cells. The transfected 293Ts were then infected with wild-type CCHFV either one day post transfection or at the same time as the transfection. We also infected 293T cells and then transfected in the S-GFP fusion RNAs 2 days post-infection.

Some of the transfected library RNAs may be packaged by the replicating wild-type CCHFV, and if the GFP fusion is in a region that does not disrupt the function of the nucleoprotein, we expect to generate recombinant CCHFV in which the wild-type S segment has been replaced with the GFP-tagged S segment. In order to select stable GFP fusions, supernatants from the infections were passaged blindly two times onto uninfected 293T cells.

Results:
We are presently screening the transfection/infection supernatants by RT-PCR to identify locations in CCHFV-S that will tolerate GFP fusions. We will then generate recombinant CCHFV-S RNA with GFP fusions in the identified locations, and repeat the process of transfection/infection. Transfecting a single recombinant RNA containing a stable GFP fusion (as opposed to the heterogeneous mix of RNAs in the initial library screen) should increase the proportion of GFP-tagged CCHFV in infection supernatants. GFP-tagged CCHFV will be purified by plaque assay or limiting dilution.

3. Key research accomplishments.

Our key research accomplishments are listed under **Conclusions** (Section 5) and in the four papers and four 2007 abstracts (listed under **Reportable Outcomes** (Section 4).

4. Reportable outcomes.

Papers and abstracts that have resulted from this research project are listed below.

PAPERS

Bertolotti-Ciarlet, A., Smith J., Strecker K., Paragas J., Altamura L., McFalls J., Frias-Staheli N., García-Sastre, A., Schmaljohn, C., and Doms, RW. (2005) Cellular localization and antigenic characterization of Crimean-Congo hemorrhagic fever virus glycoproteins. J.Virol. 79: 6152-61.

Ahmed, A., McFalls, J., Hoffmann, C., Filone, C.M., Stewart S. M., Paragas, J., Khodjaev S., Shermukhamedova D., Schmaljohn, C.S., Doms, R.W., and Bertolotti-Ciarlet, A. (2005) Presence of broadly reactive and group-specific neutralizing epitopes on newly described isolates of Crimean-Congo hemorrhagic fever virus. Journal of General Virology. 86:3327-36.

Altamura, L.A., A. Andrea Bertolotti-Ciarlet, J. Teigler, J.J. Paragas, C.S. Schmaljohn and R.W. Doms (2007). Identification of a novel C-terminal cleavage of Crimean-Congo hemorrhagic fever virus PreGN that leads to generation of an NSM protein. J. Virol. 81:6632-6642.

N. Frias-Staheli, N. V. Giannakopoulos, M. Kikkert, S. L. Taylor, A. Bridgen, J. J. Paragas, J. A. Richt, R. R. Rowland, C. S. Schmaljohn, D. J. Lenschow, E. J. Snijder, A. García-Sastre* & H. W. Virgin IV*: Ovarian yumor (OTU)-domain containing viral proteases evade ubiquitin- and ISG15-dependent innate immune responses. Cell, Host and Microbe, in press. *Corresponding authors.

ABSTRACTS TO MEETINGS (2007 only)

Altamura, L.A., Schmaljohn, C.S., and Doms, R.W. (2007) Crimean-Congo hemorrhagic fever virus encodes an NSM protein. American Society for Virology 26th Annual Meeting. July 14-18 Corvallis, OR. Oral presentation.

N. Frias-Staheli, N. V. Giannakopoulos, C. S. Schmaljohn, H. W. Virgin IV, A. García-Sastre (2007). The OTU Domain-containing L protein of Crimean Congo Hemorragic Fever Virus Has De-ISGylating and De-Ubiquitinating activities. American Society for Virology 26th Annual Meeting. July 14-18 Corvallis, OR. Oral presentation. Awarded with a prize for best presentation.

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5. Conclusions.

The major conclusions reached during the third year of this project are as follows:

A. The OTU-like domain of the L protein has isopeptidase activity and is able to overcome antiviral pathways regulated by ubiquitin and by the IFN-inducible ubiquitin-like molecule ISG-15. This activity is common with the nsp2 protease of arteriviruses.
B. The 3' UTR of the L gene of CCHFV has an unusual G at position 10, not shared by the M and S genes.
C. The Gn protein undergoes a C-terminal cleavage event that liberates an NSm protein. Cleavage appears to occur within the second transmembrane domain, making this an intramembranous cleavage event.
D. Our evidence is most consistent with signal peptidase being responsible for NSm cleavage, but we have not proven this.
E. The NSm protein is stable, transported to the Golgi, and is produced in virus infected cells. When it is genetically ablated, the viral glycoproteins do not fold and assemble correctly.
F. Codon optimization of the CCHFV M segment makes it possible to express CCHFV glycoproteins at the cell surface.
G. Cells expressing the CCHFV glycoproteins mediate fusion with other cells quickly and efficiently following low pH treatment, thus providing us with a high throughput assess to study CCHFV glycoprotein function.
H. CCHFV has been successfully passaged 18 times in SCID mice.
I. CCHFV replicates in non-human primates.

6. References.

NONE

7. Appendices.

NONE